

Presence of Beta-Lactam Antibiotic Residues in Raw Milk Obtained From Afyonkarahisar Province[#]

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ABSTRACT

Antibiotics usage for treating, protective, and similar purposes of animals may cause residue in foods of animal origin. Raw milk has high risk in terms of antibiotics residue. The consumption of such foods can cause serious health problems in human. For this purpose, 80 raw milk samples were collected from Afyonkarahisar province and analysed for beta-lactam antibiotics by means of ELISA. The result of analyses showed that 38 samples did not contain beta-lactam residue, 35 samples contained different concentration and acceptable maximum residue limits of beta-lactam, and 7 samples contained residues above 3 ppb but below 30 ppb. In conclusion, relatively high level of residue in raw milk showed that milk is offered for human consumption without taking it into consideration of withdrawal time.

Key words: Antibiotic, beta-lactam, residue, milk

Afyonkarahisar Bölgesinden Toplanan Çiğ Sütlerde Beta-Laktam Grubu Antibiyotik Kalıntı Varlığının Araştırılması

ÖZ

Hayvanlarda tedavi edici, koruyucu ve benzeri amaçlarla kullanılan antibiyotikler bu hayvanlardan elde edilen gıdalarda kalıntı bırakabilmektedir. Antibiyotik kalıntıları bakımından çiğ süt yüksek risk içermektedir. Böyle gıdaların insanlar tarafından tüketimi ise ciddi sağlık sorunlarına neden olabilmektedir. Bu amaçla, Afyonkarahisar bölgesinden toplanan 80 çiğ süt örneği beta-laktam antibiyotik kalıntı varlığı bakımından ELISA yöntemiyle analiz edildi. Yapılan analizler neticesinde 38 örneğin beta-laktam grubu antibiyotik kalıntısı içermediği, 35 örneğin değişen konsantrasyonlarda ve bulunmasına izin verilen maksimum kalıntı limitleri içerisinde ve 7 numunenin ise 3 ppb'nin üzerinde fakat 30 ppb'nin altında beta-laktam antibiyotik kalıntısı içerdiği tespit edildi. Sonuç olarak, çiğ sütlerde saptanan değerlerin nispeten yüksek olmasının antibiyotik verilmiş hayvanlardan gerekli arınma süresi geçmeden sütlerin tüketime verildiği şeklinde değerlendirildi.

Anahtar Kelimeler: Antibiyotik, beta-laktam, kalıntı, süt

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INTRODUCTION

Milk plays a significant role in children and infants nutrition. However, intensive and inappropriate use of antibiotics for the treatment of milk producing animals can result in the high prevalence of antibiotic residues in milk which can cause antibiotic resistance in several pathogenic bacteria. Antibiotic residues in milk could cause undesirable effects on human health (Kaya and Filazi 2010; Zhu et al. 2013; Acaroz et al. 2015).

Some studies showed that milk and its products could be contaminated with veterinary drugs, toxins, and other chemical agents (Kaya and Filazi, 2010; Filazi et al. 2010; Kara and Ince, 2014; 2016; Yu et al., 2017). Cristina et al. (2010) performed a study in 90 cow milk and 65 beef samples regarding macrolide antibiotic residues, in Spain. According to this study, tilmicosin, tylosin, spiramycin, and lincomycin residues were found in 44 of 90 milk samples. Han et al. (2013) collected 180 ultra-high temperature processing (UHT) milk samples from 25 different cities and markets of China. Milk samples were examined regarding antibiotic residue and sulfamethazine, sulfonamide, and quinolone antibiotic levels were found to be high level except for tetracyclines. In another study, 100 raw, 50 pasteurised, and 50 UHT (totally, 200 milk samples) milk sold in Ankara market evaluated regarding antibiotic residue and 5.5 % of milk samples were penicillin positive (Ceyhan and Bozkurt, 1987). Also, 50 raw milk samples collected from various dairy and dairy plants in

Konya. These milk samples were evaluated regarding penicillin G, ampicillin, and penicillin V residues by High Performance Liquid Chromatography (HPLC) and six milk samples were found to have antibiotic residues (Demet et al., 1992).

In this study, it was aimed to monitor the presence of antibiotic residues in milk. For this purpose, beta-lactam group antibiotic residues were investigated using Enzyme-Linked Immunosorbent Assay (ELISA) method in 80 raw milk samples collected from Afyonkarahisar.

MATERIAL and METHOD

In this study, beta-lactam-derived antibiotic residues were investigated in raw milk in Afyonkarahisar, Turkey. For that purpose, 80 milk samples were collected after milking from the various dairy plants between August-October in 2015 (Table 1). The milk samples were taken into the sterile plastic tubes, immediately brought to the laboratory and were kept at -20 °C until analysis.

5 ml of milk samples were put into each tube and centrifuged at 2000 rpm for 15 minutes. After centrifugation, the upper creamy layer was completely removed. The defatted supernatant obtained and was used for the analysis. Randox beta-lactam ELISA kit was used for the analysis. The compounds of the beta-lactam ELISA kit and the cross-reactivity were given in Table 2.

Table 1. Districts and centers where milk samples were collected

Tablo 1. Süt numunelerinin alındığı ilçe ve merkezler

Districts	Centers
City Center	Çayırbağ, Çakırık, Gebeceler, Fethibey, Erkmen, Susuz, Çukurköy, Saraydüzü, Karşıyaka, Sahipata, Nuribey, Sülün, Değirmendere, Sülümenli, Büyükkalecik Beyyazı,
Sinanpaşa	Akören, Çobanozu, Garipçe, Saraycık, Tınaztepe
Çay	Çayırpınar, Karacaören, Deresine, Bahçelievler, Göcen
İhsaniye	Gazlıgöl, Karacaahmet, Ayazini, Cumalı, Döğer, Orhanlı
Şuhut	Koçyatağı, Senir, Taşlıca, İsalı
Evciler	Altınova, Kayalık

Table 2. Beta-lactam ELISA kit compounds and their cross reactivity

Tablo 2. Beta-laktam ELISA kitinin bileşikleri ve çapraz reaktivitesi

Compound	Cross Reactivity (%)
Nafcillin	144
Ampicillin	100
Amoxicillin	94
Piperasilin	92
Azlocillin	89
Cloxacillin	69
Penicillin G	56
Dicloxacillin	52
Oxacillin	51
Metampicillin	30
Pencillin V	22
Carbenicillin	8
Tricarcillin	6
Cephalosporins	<0.01

RESULTS

At the end of the analysis, standard absorbance values were read and the concentration-absorbance curve shown in Figure 1. Absorbance values of samples were evaluated according to the standard curve. The beta-lactam residue concentration ranges and positive results of 80 milk samples were given in Table 3.

It was found that 38 samples contained no residues, 35 samples were within the maximum residue limits, and 7 samples contained residues above 3 ppb but below 30 ppb. Antibiotic contamination rate was determined as 8.75 % according to the total number of samples.

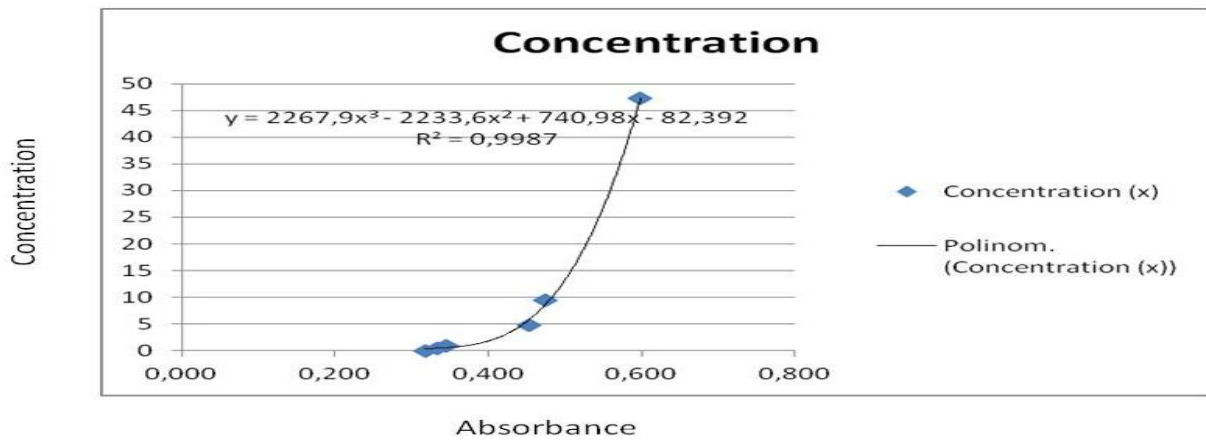


Figure 1. Concentration- Absorbance Standard Curve

Şekil 1. Konsantrasyon- absorbans standart eğrisi

Table 3. Beta-lactam antibiotic residue levels in milk samples
Tablo 3. Süt numunelerindeki beta-laktam antibiyotik kalıntı düzeyleri

Concentration (ppb)	Number of positive samples (n: 80)
0	38
0.1 - 0.5	19
0.5 - 1	8
1 - 3	8
3 - ≤30	7

DISCUSSION

Antimicrobial drugs are employed for the protection and/or treatment of animals against diseases. In addition to the desired effects of drugs and other substances, it should not be overlooked that they may also pose a risk, especially in terms of public health. The most significant of these risks is the residues of these substances. In particular, this situation is vital important for the drugs which have carcinogenic, teratogenic or mutagenic properties. At the same time, those who consume residue-containing products could have a drug allergy and inhibition of the digestive tract bacterial community. Drug residues in animal products have adverse effects regarding consumer health such as allergies and cancer. To analyse the residues of animal products has become increasingly important (Kaya and Unsal, 2000).

In the present study, beta-lactam antibiotic residues were investigated in raw milk samples consumed in Afyonkarahisar by ELISA method. As a consequence of the analysis, no residue was found in 38 samples from the 80 milk samples, in 35 samples beta-lactam antibiotic residues were detected in varying concentrations within the maximum permissible residue limits. In addition to this, beta-lactam antibiotic residues in 7 milk samples were detected among to 3 and 30 ppb.

In a survey, beta-lactam residues were investigated qualitatively in the milk samples consumed in Şanlıurfa, 64 of 300 milk samples were found to contain beta-lactam antibiotic residues, and 32 samples had other antimicrobial activity residues (Ardıç and Durmaz, 2006).

Temamoğulları and Kaya (2010) conducted a residue analysis regarding ampicillin, amoxicillin, danofloxacin, enrofloxacin, erythromycin, florfenicol, and cloxacillin in 120 pasteurise and

120 raw milk samples from 7 commercial companies. For the residue analysis, thin layer chromatography and microbiological disc diffusion technique based on bioautography were used. According to the results, ampicillin residue was detected in 1 pasteurised milk sample and it was not detected any antibiotic residue in 239 samples.

Kaya and Filazi (2010) analysed penicillin G, oxytetracycline, gentamycin, streptomycin, and neomycin antibiotic residues totally 240 raw milk samples and pasteurised milk products sold in Ankara. 1 sample of the pasteurised milk samples had 150.4 µg/L oxytetracycline, 1 sample of the pasteurised milk samples had 33.5 µg/L penicillin G and 1of the raw milk sample showed 768.4 µg/L neomycin and these concentrations of the residues in milk were above the maximum permissible residue limits in Turkey and European Union countries.

Torlak et al. (2012) performed a study to determine the performance of 3 different antibiotic test kits based on various principles was evaluated in artificially contaminated raw milk samples regarding beta-lactam antibiotics such as penicillin G, ampicillin, amoxicillin, and cloxacillin. Detection limits of HPLC method were detected as 8 µg/kg for ampicillin, 8 µg/kg for amoxicillin, 6 µg/kg for penicillin G, and 11 µg/kg for cloxacillin. In this study, the detection limits obtained by the chromatographic method were found to be above the maximum residue limits of Turkey and the European Union regarding ampicillin, amoxicillin, and penicillin G. In addition, 75 raw milk and 14 pasteurised milk of 89 milk samples, from 7 commercial firms and 9 different milking units, were evaluated in terms of chloramphenicol residues by thin layer chromatography/bioautographic method and chloramphenicol residues detected in 6 milk samples (0.8-1.6 ppm) (Şanlı et al., 1991).

In a study performed with 150 raw milk samples, collected in and around Bursa, were evaluated regarding chloramphenicol residues and it was detected in 2 milk samples (Dokuzlu and Tayyar, 2000). Similarly, in 61 milk samples collected from a various dairy plant in Konya evaluated regarding chloramphenicol residue and it was detected in 28 milk samples by HPLC (Demet et al., 1992).

Önal et al. (1993) performed a study to detect chloramphenicol residue by using intertest and triple plaque test in 444 raw and pasteurised milk obtained from public and private sector enterprises in Ankara, 78 positive (17.56 %), 65 suspected (14.63 %), 301 negative (67.79 %) results were found by intertest method and 24 positive (5.40 %), 1 suspected (0.22 %) and 419 negative (94.36 %) results were found by triple plaque method performed with *B. subtilis*.

According to the "Regulations of Pharmacological Active Ingredients and Maximum Residue Limits Regulation in Animal Husbandry" in the Turkish Food Codex, maximum residue limits of beta-lactam for penicillin derivative drugs in milk such as ampicillin, amoxicillin, and benzylpenicillin are determined as 3 ppb, for cloxacillin, dicloxacillin, nafcillin, and oxacillin as 30 ppb. Maximum residue limit for cephalosporins in milk is determined as 50 ppb (TGGK, 2007). Obtained data from our study showed that 7 samples were above 3 ppb and it was determined that beta-lactam antibiotics residue in the milk might have potential risk. This can be interpreted as the consumed milk is obtained from antibiotic given animals without waiting the withdrawal period of antibiotics.

As a result, breeders should pay attention to withdrawal period of antibiotics, they should be educated and samples from animal need to be taken and regularly controlled regarding human and living organisms health.

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